UNIVERSITI PUTRA MALAYSIA

BIOLOGICAL ACTIVITIES AND FLAVONOID CONTENT OF DIFFERENT PARTS OF Phaleria macrocarpa (SCHEFF.) BOERL FRUIT EXTRACTS

RUDI HENDRA

BIOLOGICAL ACTIVITIES AND FLAVONOID CONTENT OF DIFFERENT PARTS OF *Phaleria macrocarpa* (SCHEFF.) BOERL FRUIT EXTRACTS

RUDI HENDRA

MASTER OF SCIENCE
UNIVERSITI PUTRA MALAYSIA
2010

BIOLOGICAL ACTIVITIES AND FLAVONOID CONTENT OF DIFFERENT PARTS OF *Phaleria macrocarpa* (SCHEFF.) BOERL FRUIT EXTRACTS

By

RUDI HENDRA

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

Feburary 2011

DEDICATION

I present this thesis to my beloved father (Alm. H. Syamsuddin My.), mother (Hj. Rosni, Sy.), brothers and sister (Afrizal, Budi, Yoel, Meri, Lisa), my niece and nephew (Reza, Dinda, Dila, Yuri) for their love, patience, and understanding.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

BIOLOGICAL ACTIVITIES AND FLAVONOID CONTENT OF DIFFERENT PARTS OF *Phaleria macrocarpa* (SCHEFF.) BOERL FRUIT EXTRACTS

by

RUDI HENDRA

December 2010

Chairman : Syahida Ahmad, PhD

Faculty : Biotechnology and Biomolecular Sciences

Phaleria macrocarpa (Scheff.) Boerl (Family Thymelaceae) posses several bioactivities and is used as traditional medicine in Indonesia. Investigation on antioxidant, anti-inflammatory and antimicrobial activities are still lacking. The aims of this study were therefore to determine those biological activities of methanolic, ethyl acetate, hexane and hot water extract from pericarp, mesocarp, and seed of *P. macrocarpa* fruit and quantitative analysis of total phenolics and flavonoids present in the various bioactive extracts. The methanolic, ethyl acetate and hexane extracts of pericarp showed free radical scavenging activity (71.9±1.9, 88.1 ± 2.5 and 78.2± 1.7%) at concentration of 300 µg/ml and these results were lower as compared to butylated hydroxytoluene (BHT), ascorbic acid and α-tocopherol (antioxidant standards). The methanolic, ethyl acetate and hot water extracts of pericarp showed high reducing power with values 92.5±0.1, 84.7±0.3 and 91.9±0.1%, respectively at

300 µg/ml. All parts of *P. macrocarpa* fruit showed low to moderate level of nitric oxide inhibitory activity in IFN-γ/LPS activated RAW 264.7 cell line with values of 15.6±1.0% to 75.0±0.7% at concentration of 200 µg/ml without cytotoxic to the cells. In addition, methanol and ethyl acetate extract of *P. macrocarpa* fruits at 1.5 and 3 mg/disc showed variable inhibitory activity against both Gram-positive and Gram-negative bacteria with inhibition zone diameters ranging from 0.32-2.33 cm exclusive of paper disc (0.6 cm). While, ethyl acetate extract of *P. macrocarpa* fruits at 3 mg/well showed variable inhibitory activity against all fungi tested with inhibition zone diameters ranging from 0.4-2.13 cm exclusive of well diameter (0.6 cm).

The total phenolic contents analysis showed that ethyl acetate extract of seed contained the highest amount of total phenolic at 60.94±0.81 mg GAE/g DW while the lowest amount was observed in hexane extract of pericarp at 32.97±0.69 mg GAE/g DW. Furthermore, methanolic extract of pericarp contained the highest amount of total flavonoids with a value of 161.35±1.59 mg rutin equivalent/g DW and the lowest was observed in hexane extract of seed with a value of 9.66±0.07 mg rutin equivalent/g DW. Quantitative analysis of flavonoid compounds from *P. macrocarpa* fruit (pericarp, mesocarp and seed) were performed by using reversed-phase high performance liquid chromatography (RP-HPLC) technique. The results showed that kaempferol, myricetin, naringin, quercetin and rutin were present in all different part of *P. macrocarpa* fruit. Thus, the present results showed that *P. macrocarpa* fruit (pericarp, mesocarp and seed) poses antioxidant, anti-inflammatory and antimicrobial activities might be due to the present of flavonoid and phenolic compounds.

Abtrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI BIOLOGI DAN KANDUNGAN FLAVONOID TERHADAP EKSTRAK BAHAGIAN BUAH *Phaleria macrocarpa* (SCHEFF.) BOERL

Oleh

RUDI HENDRA

Desember 2010

Pengerusi : Syahida Ahmad, PhD

Faculti : Bioteknologi dan Sains Biomolekul

Phaleria macrocarpa (Scheff.) Boerl mempunyai beberapa bioaktiviti dan digunakan di dalam perubatan tradisional di Indonesia. Namun begitu, aktiviti antioksidan, anti-keradandan dan antimicrobial tumbuhan ini masih kurang di laporkan. Tujuan penyelidikan ini dijalankan adalah untuk menentukan aktiviti biologi tumbuhan ini di dalam pelarut metanol, etil asetat, heksana dan air panas menggunakan ekstrak pericarp, mesocarp dan biji di dalam buah *P. macrocarpa* dan analisis kuantitatif terhadap kehadiran flavonoids di dalam ekstrak yang berbeza. Ekstrak pericarp di dalam pelarut metanol, etil asetat dan hesana menunjukkan keupayaan memerangkap radikal bebas adalah (71.9±1.9, 88.1 ± 2.5 dan 78.2± 1.7%) pada kepekatan 300 µg/ml. Keputusan ini menunjukkan ianya lebih rendah daripada hidroksitoluena butilat (BHT), asid askorbik and α-tokoferol. Begitu juga dengan ekstrak pericarp di dalam pelarut metanol, etil asetat dan air panas yang menunjukkan kekuatan aktiviti kuasa penurun dengan nilai 92.5±0.1, 84.7±0.3 dan 91.9±0.1% pada kepekatan 300 µg/ml. Di dalam ujian Griess, kesemua bahagian di dalam buah *P. macrocarpa*

menunjukkan paras rendah hingga tertinggi pada aktiviti perencatan nitrik oksida pada sel RAW 264.7 dengan nilai 15.6±1.0% to 75.0±0.7 pada kepekatan 200 µg/ml dan semua ekstrak menunjukkan tiada sitotoksik terhadap sel. Selain itu, pengujian antibakteria ekstrak buah di dalam pelarut metanol dan etil asetat yang berkepekatan 1.5 dan 3 mg/well menunjukkan kadar perencatan yang pelbagai terhadap bakteria Gram-positif dan Gram-negatif dimana kadar zon diameter perencatan di antara 0.32 – 2.33 cm. Di dalam pengujian antifungal, ekstrak buah *P. macrocarpa* di dalam pelarut etil asetat yang berkepekatan 3 mg/well juga menunjukkan kadar perencatan yang pelbagai kepada semua fungi yang diuji dengan kadar zon diameter perencatan diantara 0.4-2.13cm. Di dalam penyelidikan ini, ujian kandungan fenolik menunjukkan ekstak biji di dalam pelarut etil asetat adalah paling tinggi 60.94±0.81 mg GAE/g DW manakala yang paling rendah adalah ekstrak pericarp di dalam pelarut heksana (32.97±0.69 mg GAE/g DW). Selain itu, kandungan flavonoid di dalam pericarp yang dilarutkan di dalam pelarut metanol adalah yang tertinggi iaitu 161.35±1.59 mg persamaan rutin /g DW dan kandungan flavonoid yang terendah adalah daripada ekstrak biji di dalam pelarut heksana iaitu 9.66±0.07 mg persamaan rutin /g DW. Analisis kuantitatif terhadap flavonoid daripada buah *P. macrocarpa* (pericarp, mesocarp dan biji) di uji dengan menggunakan teknik kromatografi fasa-pembalikan HPLC. Keputusan menunjukkan kemferol, mirisetin, naringin, kursetin dan rutin merupakan flavonoid yang hadir di dalam bahagian yang berbeza di dalam buah *P. macrocarpa*. Dengan demikian, hasil kajian menunjukkan aktiviti antioksidan, anti-inflamasi dan antimikrob yang mungkin berkaitan dengan sebatian flavonoid dan fenol dalam ekstrak mentah.

ACKNOWLEDGEMENTS

First and foremost, thank and praised God, for making this research an unforgettable one, and for the time to finish it. I would like to express my deepest gratitude and appreciation acknowledgment to each and every one who has contributed in finalizing this thesis. Special thanks go to Dr. Syahida Ahmad, Associate Professor Dr. Radzali Muse, my main supervisor, for their patience, guidance, advice and their continuous support toward the completion of my thesis. I also wish to thank the other members of my supervisory committees, Professor Dr. Mohd. Aspollah Hj. Md. Sukari and Associate Professor Dr. Mohd. Yunus Abd Shukor for their guidance and valuable review to this research. I am sincerely thankful and greatly appreciative for all of the help, encouragement, guidance and friendship that I have gained during this project from the staff of the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences and research assistants of animal tissue culture laboratory, Institute of Biosciences, Universiti Putra Malaysia. I also hereby to knowledge, with gratitude, rector, dean, colleague in Faculty of Mathematic and Natural Sciences, University of Riau for their support and gave me opportunity to continue my study. Not forgetting to Indonesia student association (PPI-UPM), my housemate (R719) for their friendship in these couple years, Ehsan Oskoueian for his friendship, guidance, and patience, Mr. Mohd Fadhil for his help on the high performance liquid chromatography (HPLC) techniques, Chow Yuh Lit for her help on the animal tissue culture techniques. Last but not least, I want to thank to my family and friends (Heri, Teza, Amar, Rizky, Yulia, Gita, Nisa, Ayu, Mariam, Dayana, Azah) for their love, support and encouragement throughout my study.

I certify that an Examination Committee has met on date/month/year to conduct the final examination of Rudi Hendra on his Master of Science thesis entitle "Biological activities and flavonoid content of different parts of *Phaleria Macrocarpa* (scheff.) Boerl fruit extracts" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science.

Members of the Examination Committee were as follows:

Mohd Puad Abdullah, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
University Putra Malaysia
(Chairman)

Norhani Abdullah, PhD
Professor
Faculty of Biotechnology and Biomolecular Sciences
University Putra Malaysia
(Internal Examiner)

Farida Abas, PhD
PhD
Faculty of Food and Technology
University Putra Malaysia
(Internal Examiner)

M.S. Kanthimathi, PhD
Associate Professor
Department of Biomedic/ Faculty of Medicine
University of Malaya
Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 22 February 2011

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Syahida Ahmad, PhD
Senior lecturer
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Mohd. Aspollah Hj. Md. Sukari, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Member)

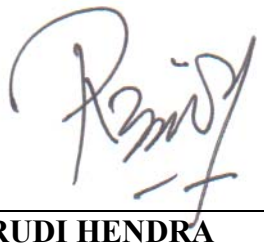
Mohd. Yunus Abd Shukor, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



RUDI HENDRA

Date: 17 December 2010

TABLE OF CONTENTS